# Tumor Suppressor MicroRNAs: a novel non-coding alliance against cancer

Giovanni Blandino [1*], Francesco Fazi [2-3], Sara Donzelli[1], Merav Kedmi[4], Aldema Sas-Chen[4], Paola Muti[5], Sabrina Strano[6], and Yosef Yarden[4].

* Corresponding author

1. Translational Oncogenomics Unit, Italian National Cancer Institute 'Regina Elena', Rome, Italy

2. Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina 04100, Italy

3. San Raffaele Bio-Medical Park Foundation, Rome 00128, Italy

4. Weizmann Institute of Science, Department of Biological Regulation, Rehovot, Israel

5. Department of Oncology, Juravinski Cancer Center-McMaster University Hamilton, Ontario, Canada

6. Molecular Chemoprevention Unit, Italian National Cancer Institute 'Regina Elena', Rome, Italy

**Abstract**

Tumor initiation and progression are the outcomes of a stepwise accumulation of genetic alterations. Among these, gene amplification and aberrant expression of oncogenic proteins, and deletion or inactivation of tumor suppressor genes, represent hallmark steps. Mounting evidence collected over the last few years identifies different populations of non-coding RNAs as major players in tumor suppression in almost all cancer types. Elucidating the diverse molecular mechanisms underlying the roles of non-coding RNAs in tumor progression might provide illuminating insights, potentially able to assist improved diagnosis, better staging and effective treatments of human cancers. Here we focus on several groups of tumor suppressor microRNAs, whose downregulation exerts a profound oncologic impact and might be harnessed for the benefit of cancer patients.

## Introduction

In most epithelial tissues, cancer develops through separate and interrelated steps of clonal expansion, genetic diversification, and clonal selection. During cancer development, cancer cells acquire diverse biological capabilities that are conferred by the numerous genetic and epigenetic modifications [1]. In recent years, different high-throughput platforms have been used for the genomic, transcriptomic, proteomic, and epigenomic analyses to search for new biomarkers involved in cancer and to bring new insights into the several aspects of cancer pathophysiology [1]. In addition to the classical transcriptional cell regulators involved in cancer development, a class of noncoding RNAs, termed microRNAs (miRNAs) has emerged as critical regulators of gene expression acting predominantly at the post-transcriptional level. MiRNAs were first identified through their ability to regulate developmental processes, such as developmental timing and cell fate transitions [2]. Subsequently, miRNAs have been studied in relation to cancer development. A large number of miRNAs that map to specific regions of the human genome have been shown to be frequently deleted or amplified in cancer [3]. Several lines of evidence indicate that miRNAs might be differentially expressed in cancer cells, in which they forme unique expression patterns or signature [4]. Sevignani and colleagues reported a significant association between the chromosomal location of miRNAs and those of mouse susceptibility loci that influence the development of solid tumors [5]. Dysregulation of miRNAs in cancer can occur through both epigenetic changes, including aberrant DNA methylation and histone modification [6], and genetic alterations. These two biological mechanisms can affect the production of the primary RNAs, their processing to the mature miRNA forms, and/or interactions with mRNA targets [7].

More recent studies indicate that mutations affecting proteins involved in the processing and maturation of miRNA, such as TARBP2, DICER1 and XPO5, can also lead to overall reductions in miRNA expression [8-10]. Consistent with these observations, miRNAs are thought to act mainly

as tumor suppressor genes, and their dysregulation is currently recognized as a common feature of human cancers. Later on, additional data indicated that the expression of miRNAs is mainly downregulated in tumor tissues, as compared to corresponding healthy tissues, which supported the role of miRNAs as primarily tumor suppressors [4, 8, 9, 11, 12]. In the same vein, there is evidence that an extensive down-regulation of miRNAs is one of the first outcomes of the stimulation of signaling cascades downstream to specific growth factor receptors implicated in a number of human cancers, including breast cancer [13]. For example, EGF signaling rapidly and simultaneously induces an extensive down-regulation of multiple microRNAs, reflecting coordinated regulation at the level of microRNA synthesis, processing or degradation [13].

MiRNAs are also deregulated upon exposure to both metabolic cancer risk factors and exposures to carcinogenic substances [14, 15]. Thus, miRNAs may represent at the same time both predictors and players of cancer development. A number of life-style factors (e.g., diet rich in fats and refined carbohydrates) and pathological conditions (e.g., obesity), often related to inflammation and cancer, result in deregulation of specific microRNAs [16-19]. There is evidence of an altered expression of miRNAs in relation to the exposure to well-known carcinogenic substances such as asbestos, formaldehyde and cigarette smoke in lung and hepatic tissue [20, 21]. In regard to this evidence, one study examined the expression of 484 miRNAs in the lungs of rats exposed to environmental cigarette smoke for 4 weeks. It was found that 126 miRNAs were down-regulated at least 2-fold and 24 miRNAs were downregulated more than 3-fold [22].

In this review, we highlight the contribution of miRNA modulation, in particular prevalent downregulation of specific miRNAs, to cancer development. Because many downregulated miRNAs function as tumor suppressors, better understanding of the biological mechanisms underlying their modulation will enable new strategies for early detection, prevention and therapy of cancer.

**MicroRNA-10b3p: the early arm of the microRNA-10b locus**

The so called microRNA-10b locus is located on chromosome 2, within the cluster of the HOXD genes, in an intergenic region between HOXD4 and HOXD8 [23]. Processing by Drosha and Dicer, transforms the RNA product of the microRNA-10b locus into a 22-nucleotide RNA duplex that contains two distinct 5' phosphorylated strands with 3' overhangs (Figure 1). The functional strand of the duplex, referred to as the guide strand, is microRNA-10b5p while the other, passenger strand generates microRNA-10b3p. MicroRNA-10b5p was originally identified as a molecules down-regulated in primary breast tumors, compared to normal breast tissues [24]. Similarly, down-regulation of microRNA 10b5p by promoter hypermethylation has been reported in gastric tumors [25]. The Weinberg's group has later reported that microRNA-10b5p acts as a metastasis-supporting microRNA, due to its ability to favor migration and invasion of breast cancer cells [26, 27]. In line with this, microRNA-10b5p targets the HOXD10 gene, a repressor of several modulators of cell migration [26]. The expression of microRNA-10b5p is tightly controlled by the transcription factor Twist, a well-established regulator of epithelial-to mesenchymal transition (EMT). Increased expression of microRNA-10b5p was detected in the vasculature of breast IDC (invasive ductal carcinoma) grade III tumors compared to lower expression in DCIS (ductal carcinoma in situ) [28]. The pleiotropic activity of microRNA-10b5p could also rely on its ability to target the expression of diverse tumor suppressors including TP53, HODX10, PAX6, NOTCH1 and FOXO3 (see Table 1) [29] .

Biagioni et al. originally reported that the expression of microRNA-10b3p was down-regulated in breast tumors, relative to matched peritumoral tissues [12]. This down-regulation occurred, at least in part, through the methylation of CpG islands located within the regulatory regions of the microRNA-10b locus [12]. Ectopic expression of microRNA-10b3p inhibited proliferation of breast cancer cell lines and reduced the size of xenografted breast tumors [12]. Three pivotal proteins involved in the control of cell proliferation, namely BUB1, PLK1, and

CCNA2, were shown to serve as targets of microRNA-10b3p. Accordingly, intratumoral injection of a mimic of microRNA-10b3p reduced the expression of BUB1, PLK1 and CCNA2 proteins [12]. The prognostic role of microRNA-10b3p and of its target was evaluated in the MEATBRIC dataset. This analysis included 1,286 breast cancers from 5 different subtypes: HER2+ (127 patients), basal-like (209 patients), luminal A (479 patients), luminal B (312 patients), and normal-like (151 patients) and for which both mRNA and microRNA data were availble [12]. mRNAs and miRNAs were measured for each tumoral and normal samples of the METABRIC dataset. Kaplan-Meyer analysis revealed a significant association between low expression levels of miR-10b3p and poor disease-specific survival [12]. This association was not evidenced for the augmented expression of microRNA-10b5p. The combined application of COSMIC algorithm [30] and miRanda predictions uncovered 15 target mRNAs of microRNA-10b3p [12]. Among those targets there, BUB1, PLK1 and CCNA2 were confirmed, and additional cell cycle related targets were also identified. Increased expression of BUB1, PLK1 and CCNA2 was associated with poor survival (Table 1) [12].

These findings have several implications to the roles played by microRNA-10b in breast tumorigenesis. Presumably, the expression of microRNA-10b3p is altered in the early stages of mammary cell transformation. This could lead to aberrant cell proliferation, mediated by increased expression of the cell cycle related targets of microRNA-10b3p. In line with early alterations, down-regulation of microRNA-10b3p expression appears to occur independently from the subtype of breast cancer, suggesting that it might represent an event preceding specification of breast cancer subtypes. Interestingly, the regulation of the expression of the two strands derived from the microRNA-10b locus is controlled by the combination of epigenetic and transcriptional events. While down-regulation of microRNA10b-3p occurs through methylation of CpG islands, the transcription factor TWIST up-regulates the expression of microRNA-10b5p [26]. This might underlie mechanistically the dual and opposite roles of the microRNA-10b locus. Early in breast tumorigenesis microRNA-10b3p down-regulation leads to aberrant cell proliferation, while

TWIST-mediated transcriptional induction of microRNA-10b5p contributes, as a late step, to shape a metastatic phenotype.

Unlike down-regulation of microRNA-10b3p, which occurs independently from the breast cancer subtype, upregulation of microRNA-10b5p might be specifically selected in highly metastatic breast tumors. Thus, waves of microRNA-10b3p and 5p targets might tune the pro-proliferative and pro-metastatic activities of the aberrantly activated microRNA-10b locus. Intriguingly, microRNA-10b3p (previously named microRNA-10b*) could represent a prototype of microRNAs derived from passenger strands, which target specific mRNAs and exert biological activities as efficiently as those originated from guide strands. While the role of microRNA-10b5p is relatively well documented in different human cancers, that of microRNA-10b3p is poorly investigated. This also indicates that the microRNA-10b locus, via downregulation of the 3p strand or up-regulation of 5p plays a pivotal role in breast cancer establishment or dissemination. Once, the molecular events responsible for aberrant activation of the microRNA-10b locus in tumors will be fully understood, they might hold promise for novel therapeutic strategies. This might turn true also for passenger derived tumor suppressor microRNAs.

**Let-7c acts as a tumor suppressor microRNA**

The let-7 family is one of the most ancient and conserved groups of microRNAs, showing high conservation across species from *Caenorhabditis elegans* to mammals [31]. In humans, the let-7 family is comprised of ten members (let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-98 and miR202), which differ in their nucleotide sequences. Some of the isoforms appear in multiple copies in the genome, hence a number is added as a suffix to their name (e.g., let-7a-1, let-7a-2 and let-7a-3) (reviewed in [32]). Interestingly, many of the let-7 miRNAs are located in fragile sites and specific genomic regions that relate to cancer [3]. Thus, for example, in the human genome, the cluster let-7a-1/let-7f-1/let-7d is included in a frequently deleted region of

chromosome 9 (region B at 9q22.3). In breast carcinomas, a region of LOH (loss of heterozygosity) at 11q23 was shown to harbour the cluster miR-125b1/let-7a-2/miR-100, and the cluster miR-99a/let-7c/miR-125b-2 resides at 21p11.1, a region of frequent HD (homozygous deletions) in lung cancers. Furthermore, let-7a-1, let-7f-1, let-7d and miR-202 are located close to class II homeotic genes in the human HOX gene clusters [3].

The let-7 family plays crucial roles in cellular differentiation and in development, and it displays specific temporal and spatial expression patterns during development of several species [31, 33]. For example, in mammals, the level of let-7 increases during embryogenesis and during brain development [34]. In concordance with developmental roles for the let-7 family, members of this family are also involved in cancer, and as discussed below, many of them act as tumor suppressor miRNAs. Downregulation of most let-7 family miRNAs was demonstrated in several types of human cancer, including lung, head and neck squamous cell carcinoma, breast, melanoma, ovarian and prostate cancers. Interestingly, silencing all let-7 family members in ovarian cancer cell lines increased cell survival, invasion and adhesion [35]. The targets of let-7 are also conserved from worms to humans, and they include known oncogenic transcripts, such as MYC and RAS [36]. Interestingly, the viral homolog of KRAS contains a single nucleotide polymorphism (SNP) in its potential let-7 binding site, which increases the risk for lung, oral and colorectal cancers [37-39]. Another cancer related target of the let-7 family is the high-mobility group AT-hook 2 (HMGA2) oncoprotein, a chromatin-associated non-histone protein that affects transcription by modulating chromatin's architecture [40].

Let-7c, a member of the let-7 family, functions as a tumor suppressor in several types of cancer (Figure 2). It targets various oncogenes and cancer related genes, such as: N-RAS, C-MYC, HMGA2, MMP11, PBX2, PBX3, TRIB2, ITGB3, TGFBR1, BCL-XL and MAP4K3 [36, 37, 40-42] (see Table 2). In support of a role for let-7c as a tumor suppressor, its reduced expression was demonstrated in tumors and cultured cells from prostate, lung, colorectal and hepatocellular tumors [41, 43-46]. Additionally, downregulation of let-7c was associated with poor prognosis in colorectal

cancer, where let-7c expression was significantly lower in patients presenting lymph node involvement or distant metastases, compared to patients without any detectable metastasis [43]. In non-small cell lung carcinoma (NSCLC), low expression of let-7c was associated with poor patient survival, as well as with tumor spread, venous invasion and advanced disease stages [46]. Interestingly, let-7c is encoded by chromosomal locus 21q21, which shows loss of heterozygosity in lung cancer [47].

The maturation process of miRNAs of the let-7 family, including that of let-7c, is regulated by the RNA-binding proteins called Lin28 and Lin28B, which employ several different regulatory mechanisms [48]. Lin28 is expressed in the cytoplasm and blocks processing of let-7 by Dicer through direct binding to the terminal loop of pre-let-7 and by recruiting the terminal uridyl transferase (TUTase) Zcchc11/TUT4 to catalyze the oligouridine tail, thus marking pre-let-7 for degradation [49, 50]. Lin28B is found mainly in the nucleolus and upon binding to pri-let-7 it blocks the activity of the microprocessor complex through TUTase-independent mechanism [reviewed by [51]]. Interestingly, miRNAs of the let-7 family have been suggested to reciprocally downregulate the expression of Lin28 and Lin28B and thus increase the levels of mature let-7 family miRNAs [52, 53]. Let-7c's expression levels can also be regulated by the peroxisome proliferator-activated receptor α (PPARα) in hepatocellular carcinoma cells [45].

Manipulation of let-7c expression in cancer cell lines further established its role as a tumor suppressor. For example, overexpression of let-7c decreased, whereas depletion of let-7c increased cell proliferation and clonogenicity of prostate cancer cells *in-vitro*. Let-7c also exerted an anti-proliferative effect *in-vivo*, when tested by intratumoral injection of let-7c, which significantly reduced tumor size in xenografts of human prostate cancer cells [44]. According to recent studies, let-7c can also function as a metastasis suppressor. In highly metastatic colorectal adenocarcinoma cells, ectopic over-expression of let-7c led to reduced migration and invasion *in vitro*, and almost completely inhibited tumor growth and metastasis. When tested in weakly metastatic cells, let-7c inhibition resulted in increased cell motility and invasion. These effects of let-7c on motility were

likely mediated by targeting of KRAS, MMP11 and PBX3 [43]. Additionally, ectopic let-7c expression in chemotherapy-resistant lung adenocarcinoma cells reduced the number of metastatic nodules in lung and liver, probably via inactivation of the AKT pathway [37].

In addition to the AKT pathway, let-7c controls several other cancer-related signalling pathways. Thus, let-7c plays an important role in the regulation of androgen receptor (AR) signalling by directly targeting MYC, thereby controlling prostate cancer proliferation. Accordingly, the expression of let-7c and AR are negatively correlated in human prostate cancer, where AR, MYC and Lin28 expression levels are high while let-7c expression level is low [54]. In acute leukaemia, let7-c targets PBX2, a homeodomain protein, which upon aberrant expression enhances HoxA9-dependent leukemogenesis, and promotes granulocytic differentiation [41]. Let-7c may also suppress cell growth and control cancer pathogenesis by regulating the mitogen-activated protein kinase (MAPK) pathway. In lung adenocarcinoma let-7c directly targets TRIB2, which in turn activates the downstream components, C/EBP-α and a phosphorylated p38-MAPK [55]. Another pathway by which let-7c can regulate the MAPK pathway is through its role in controlling NRAS expression [36]. One of the key metastasis-driving processes is EMT [56], which is affected by several transcriptional switches, including a let-7c regulated switch. Docetaxel-resistant lung adenocarcinoma cells are characterized by fibroblast-like morphology and adhesion, which are typical of the mesenchymal phenotype. However, upon expression of the let-7c precursor, these cells gained an epithelial phenotype, which might contribute to the restoration of chemo- and radio-sensitivity. This effect of let-7c is possibly achieved through direct targeting of BCL-XL [37].

In summary, let-7c plays a crucial role in cancer pathogenesis through targeting key cancer-related proteins and acting both as a suppressor of both tumor growth and tumor spread. Thus, let-7c is might be considered an attractive candidate for drug-induced manipulation in cancer therapy.

**miR-223 in cell differentiation and tumor suppression.**

MiR-223 was initially identified as a miRNA nearly exclusively expressed in bone marrow, such that its functional role in the regulation of the cell fate determination of human hematopoietic progenitors cells (HPCs) rapidly emerged [57, 58]. Recently, Vian and colleagues evidenced that in human CD34+HPCs undergoing unilineage differentiation/maturation, miR-223 is up-regulated during granulopoiesis rather than during monocytopoiesis and is maintained at low levels during erythropoiesis [59]. Interestingly, miR-223 overexpression in human CD34+HPCs favors granulopoiesis and impairs erythroid and monocytic/macrophagic differentiation [59].

The fine-tuning of miR-223 expression levels during hematopoietic differentiation of HPCs, as well as of myeloid cell lines, into erythroid, granulocytic and monocytic/macrophagic lineages is the result of the coordinated recruitment and function of lineage-specific transcription factors (TFs) on two different regulatory regions of the miR-223 promoter [58, 59]. For example, during granulocytic differentiation of human myeloid progenitors, the induction of miR-223 expression is transcriptionally regulated by the competitive binding of two TFs, Nuclear Factor I (NFI-A) and CCAAT/enhanced-binding protein alpha (C/EBPα), to the proximal miR-223 regulatory region. NFI-A maintains miR-223 transcription at low levels, while its replacement by C/EBPα results in miR-223 induction and granulocytic differentiation [60]. NFI-A was also identified as a target for miR-223 at transcriptional and translational level, thus establishing a feedback regulatory circuitry in the control of granulopoiesis [58, 61].

Interestingly, miR-223 expression is down-regulated in different subtypes of acute myeloid leukemia (AML), that represents the clonal expansion of hematopoietic precursors blocked at different stages of differentiation [62]. In particular, primary blasts carrying the t(8;21) generating AML1/ETO, the most common acute myeloid leukemia-associated fusion protein, present very low levels of miR-223 expression. In these cells the AML1-ETO oncoprotein, which recruits an epigenetic silencing complex consisting of HDACs, DNMTs, and methyl-CpG-binding proteins on

the AML1 binding site of the miR-223 promoter, links the epigenetic silencing of a microRNA locus to the differentiation block of this acute myeloid leukemia subtype [62]. Of note, the enforced expression of miR-223 in primary AML blasts and in AML cell lines affects cell cycle progression and enhances granulocyte differentiation [58, 59, 62, 63].

The tumor suppressor function of miR-223 in acute myeloid leukemia is further supported by a recent study showing that the induction of miR-223 inhibits the translation of the cell-cycle regulator E2F1 and significantly down-regulates the proliferation rate of myeloid progenitors cells [63]. Of note, E2F1 transcriptionally represses miR-223 gene in AML cells, suggesting that the miR-223 function as a key regulator of myeloid cell proliferation is strictly linked with E2F1 activity in a mutual negative feedback loop [63].

Exosomes or microvesicles are emerging as important mediators of intercellular cross-talk for the regulation of a variety of biological functions, as cellular communication, proliferation and differentiation [64]. Microvesicle transfer represents a novel mechanism by which infiltrating mononuclear phagocytes may contribute to cellular activation, survival, and immune function. MiR-223 was evidenced to be the most highly expressed miRNA in the macrophage-derived microvesicles that are able to induce cellular differentiation when added to naive monocytes, supporting a functional amplification loop to enhance immune function (Figure 3A) [65].

In addition to the exosome-mediated transfer of nucleic acids, tissue macrophages were also shown to be able to transfer miR-223 to hepato-carcinoma cells (HCCs) in a manner that required intercellular contact and gap junction communication (Figure 3B) [66]. Functionally, the transfer of miR-223 from macrophages to HuH7 cells resulted in the inhibition of proliferation of these HCCs cancer cells, thus highlighting intercellular transfer of miRNA from immune cells as a new possible mechanism of defense against neoplastic cell transformation or tumor growth [66]. The transfer of miR-223 influences protein expression in HuH7 cells. Specifically, miR-223 decreases the

expression of Stathmin-1 (STMN1) and insulin-like growth factor-1 receptor (IGF-1R), which both influence cellular proliferation and can support the growth of tumors [67, 68].

STMN1 is a key microtubule-regulatory protein that controls microtubule dynamics, cell proliferation and, in particular, the S-phase of the cell cycle. High-levels of STMN1 have been associated with increased histologic grading, shorter patient survival times, and increased drug resistance in different tumors. STMN1 is a protein that is usually present at low levels in healthy hepatocytes but is expressed at high levels in hepato-carcinomas [67]. In HCC cell lines, a strong inverse relationship between STMN1 mRNA and miR-223 expression was observed and a substantial reduction in STMN1 protein was demonstrated upon restoration of miR-223 expression, resulting in a consistent inhibitory effect on cell viability [67].

In line with these lines of evidence, it was recently reported that modulation of miR-223/STMN-1 pathway represents another way by which mutant p53 increases cellular resistance to chemotherapeutic drugs [69]. The induction of mutant $p53^{R175H}$ in breast and colon cancer cell lines decreases miR-223 expression. Mutant $p53^{R175H}$, together with the transcriptional repressor ZEB-1, binds to the miR-223 promoter and decreases miR-223 expression, resulting in an up-regulation of STMN-1 and in an increased cellular resistance to chemotherapeutic agents. On the contrary, ectopic expression of miR-223 can lower the levels of STMN-1 and sensitize breast and colon cancer cell lines expressing mutant p53 to treatment with DNA-damaging drugs [69].

By targeting IGF-1R and the cyclin-dependent kinase 2 (CDK2), miR-223 functions as a potent tumor suppressor also in the Lewis lung carcinoma (LLC) cell line [70]. Ectopic expression of miR-223 suppresses proliferation, invasion and tumorigenicity of LLC cells, induces G2/M phase arrest and inhibits tumor growth in vivo, providing the basis for novel therapies targeting IGF-1R in the treatment of NSCLC [70].

The inhibition of cancer cells proliferation by miR-223 was recently reported also in colorectal cancer [71]. The colorectal cancer HCT116 cells express very low levels of endogenous miR-223 and the miR-223 overexpression in these cells reduces mRNA and protein expression

levels of FOXO1, whose abnormal expression or activation can result in aberrant apoptotic pathways, proliferation, and cell cycle regulation. Interestingly, miR-223 overexpression mainly increases the unphosphorylated FOXO1 protein, its nuclear localization, as well as cyclin D1/p21/p27 at either mRNA or protein accumulation and inhibition of tumor cell proliferation [71]. Although several experimental evidences support the involvement of miR-223 in cell differentiation and tumor suppression, miR-223 was also reported to be up-regulated in some tumors and to contribute to leukemogenesis in specific disease subtypes (T-ALL), indicating that the biologic effect of miR-223 strongly depends on the cellular context where this miRNA specifically performs its function [72, 73].

**miR-145 in tumor suppression**

miR-145 is located at chromosome 5q33.1 and is usually transcribed in a bicistronic primary transcript with miR-143 [74]. Of note, miR-145 represents one of the miRNAs that are highly expressed in normal tissues but they are down-regulated in several human cancers, including colorectal and breast cancer [24, 75]. Although the down-regulation of miR-145 expression in human cancer may result from genetic aberrations, as occurs in hematological malignancies associated to the 5q- syndrome phenotype [76], a major mechanism for the modulation of miR-145 expression is represented by transcriptional and post-transcriptional control. Interestingly, transcriptional contribution to the regulation of miR-145 expression was reported in breast and in colon cancer cell lines where p53, by interacting with a consensus sequence in the miR-145 promoter, transcriptionally induces miR-145 expression, promoting the post-transcriptional down-regulation of MYC and consequently the inhibition of tumor cell growth [77]. In line with these results, in prostate cancer tissues and cell lines, miR-145 was found to be silenced through the methylation of its promoter, and miR-145 silencing was significantly correlated to the status of the p53 gene [78]. A recent study also reported that the CCAAT/enhancer binding protein beta

(C/EBPβ) is able to counteract the p53-mediated induction of miR-145. In fact, C/EBPβ induces the transcriptional down-regulation of miR-145 expression by interacting with a CCAAT binding site located in miR-145 promoter; this down-regulation seems to be independent from p53 and involves the AKT pathway [79].

Concerning miR-143/miR-145 processing, it was recently reported that p53 itself may be involved in the post-transcriptional maturation of several miRNAs with growth-suppressive functions in response to DNA damage, including miR-143/miR-145. In particular, the p53 protein, through the association with the DEAD-box RNA helicase p68 (also known as DDX5), interacts with the Drosha processing complex thus supporting the processing of primary miRNAs to precursor miRNAs. On the contrary, p53 mutated proteins interfere with the functional assembly between the Drosha complex and p68, inducing an attenuation of miRNA processing activity [80].

Moreover, it was also recently reported that the DEAD-box RNA helicase 6 (DDX6), that is highly expressed in most malignant cells, post-transcriptionally down-regulates both miR-143 and miR-145 expression. In human gastric cancer cells, DDX6 protein, that is abundantly expressed and accumulated in processing bodies (P-bodies) containing many proteins involved in mRNA turnover, negatively regulates the RNA stability of the bicistronic primary transcript resulting in the down-regulation of both miR-143 and miR-145 [81]. Several reports, supporting miR-145's activity as a tumor suppressor in different tumor types, have revealed that the down-regulation of miR-145 expression is associated to neoplastic cell growth and proliferation, as well as to cancer cell migration, invasion and metastasis (Table 4) [82-87].

In colon cancer the tumor suppressor function of miR-145 was initially related to the down-regulation of the insulin receptor substrate-1 (IRS-1). Of note, in human colon cancer cells miR-145, by targeting the 3' UTR of IRS-1, dramatically down-regulates IRS-1 protein, thereby inducing cell growth arrest [88]. In colon cancer cell lines, YES and STAT1 factors were also evidenced as additional miR-145 targets [89]. Interestingly, in colon cancer tissues low miR-145

expression levels are inversely correlated to p70S6K1 protein levels. On the contrary, the forced expression of miR-145, by targeting p70S6K1, resulted in the down-regulation of two downstream molecules of p70S6K1 pathway, the VEGFA and HIF-1α proteins, thus inhibiting tumor growth and angiogenesis [90].

The tumor suppressive function of miR-145 on cancer cell proliferation was also recently reported in lung cancer. By targeting the EGFR and nucleoside diphosphate linked moiety X-type motif 1 (NUDT1) at both mRNA and protein levels, miR-145 inhibits lung adenocarcinoma cell proliferation and lung tumorigenesis [91]. Of note, the prognostic value of miR-145 was originally evidenced in lung cancer, where the low expression miR-145 is significantly correlated with a worse prognosis and survival of adenocarcinoma patients [92].

In breast cancer tissues, miR-145 expression is inversely correlated with the stage of malignancy. In particular, miR-145, through the post-transcriptional regulation of N-RAS and VEGFA expression, exhibits inhibitory roles in tumor angiogenesis, invasion and tumor growth [93]. Accordingly, in the human breast cancer cell line MCF-7, miR-145 induction was shown to promote the inhibition of cell growth and the induction of apoptosis by targeting the Rho-effector rhotekin (RTKN) [94]. A death-promoting loop between miR-145 and TP53 was also identified in MCF7 breast cancer cells expressing wild-type TP53. miR-145 indeed activates the p53 pathway, resulting in the promotion of apoptosis, and sustaining in turn a further induction of miR-145 expression. In the same context, miR-145 may also down-regulate estrogen receptor-alpha (ER-a) protein expression, whereby a miR-145 re-expression therapy was proposed, at least for the subgroup of patients with ER-a-positive and/or TP53 wild-type tumors [95]. Interestingly, the re-expression of miR-143/miR-145 was also shown to suppress the cellular growth and to support the apoptosis of epithelial cancer cells by enhancing p53 activity via MDM2 turnover [96].

The involvement of miR-145 in an integrated transcriptional regulatory circuit together with TFs and chromatin-modifying activities that support the growth and function of breast cancer stem-

like cells (CSCs) recently emerged [97]. Accordingly, previous results evidenced that expression of miR-145 is low in self-renewing human embryonic stem cells (hESCs) but highly up-regulated during differentiation. Increased miR-145 expression inhibits hESC self-renewal, represses expression of pluripotency genes, such as OCT4, SOX2, and KLF4, and induces lineage-restricted differentiation [98].

In malignant pleural mesothelioma (MPM) cells miR-145 has the potential to modulate many pro-tumorigenic features, including growth, clonogenicity and tumor engraftment in vivo. Interestingly, miR-145 targets directly OCT4 and, indirectly, its EMT-promoting target ZEB1, in MPM cells. Of note, the levels of miR-145 and OCT4 are inversely correlated in vivo. Promoter hyper-methylation may contribute to the low levels of miR-145 in both MPM cells and malignant MPM tissues. Importantly, miR-145 down-regulation has been proposed to classify benign versus malignant mesothelial tissues [99].

In pancreatic cancer cells, the activation of the KRAS signaling pathway consistently leads to the repression of the miR-143/miR-145 cluster and this is necessary to maintain the tumorigenic potential of these cancer cells. The down-regulation of miR-143/miR-145 expression requires the RAS-responsive element-binding protein (RREB1), which represses the miR-143/miR-145 promoter. Both KRAS and RREB1 transcripts are direct targets of these miRNAs (see Table 4), demonstrating the existence of a feed-forward pathway that potentiates KRAS-mediated tumorigenesis [100].

More recently, the RREB1 protein was found to be overexpressed also in colorectal adenocarcinoma tumors and cell lines, where the expression of the miR-143/miR-145 primary transcript is inversely related to that of RREB1. RREB1 negatively regulates expression of the miR-143/miR-145 cluster in a KRAS-dependent manner, thus establishing a complex network of regulation through which the miR-143/miR-145 cluster is able to modulate KRAS signaling [101]. In line with this, additional direct and indirect miR-143/miR-145 target genes have been reported,

and they belong to the growth factor receptor-mitogen-activated protein kinase network, as well as to the p53 signaling pathway, further supporting a contribution of miR-143/miR-145 to the cell signaling pathways involved in colorectal tumorigenesis [102].

In prostate cancer miR-145 is consistently down-regulated. A significant inverse correlation between the expression of miR-145 and that of the BNIP3 protein was observed in prostate cancer and in benign prostate tissues. Accordingly, the overexpression of miR-145 in prostate cancer PC-3 and DU145 cells significantly down-regulated BNIP3, reduced cell growth, and increased cell death. As aforementioned for breast and colon cancer, the overexpression of wild-type p53 resulted in the up-regulation of miR-145 expression also in PC-3 cells, with consequent pro-apoptotic effects [103]. Wild-type p53 induces up-regulation of miR-145 expression and the inhibitory effects of wild-type p53 on migration, invasion, EMT and stemness of PC-3 cells were reversed by anti-miR-145. These results suggest that loss of wild-type p53 may promote bone metastasis of prostate cancer, at least partially through miR-145 down-regulation, and resulting in increased EMT and stemness of cancer cells [104]. Moreover, the ectopic expression of miR-145 in LNCaP and DU145 cell lines led to a reduction in the expression of the ERG protein, suggesting that down-regulation of miR-145 associated with prostate cancer may contribute to the increased expression of several ERG isoforms that are observed in this tumor type [105].

In conclusion, several lines of evidence indicate that miR-145 may be largely considered a miRNA with tumor suppressor activity, which is involved in the regulation of tumor growth, cell invasion and metastasis by targeting multiple cancer related genes, thus offering miR-145 as a novel therapeutic target for cancer therapy.

**MicroRNA-204: a key player in development and tumor suppression**

MicroRNA-204 is encoded by the cancer associated genomic region (CAGR) 9q21.1-q22.3 locus that exhibits high frequency of loss of heterozygosity in diverse human cancers [106]. MicroRNA-204 is an intragenic microRNA, which is located within the transient receptor potential melastatin-3 (TRPM3) gene belonging to the family of transient receptor potential (TRP) channels [107].

MicroRNA-204 activity is highly involved in vertebrate lens development and its loss is evidenced in different human cancers (fig. 4). Banfi's group has shown that microRNA-204 is highly expressed in retinal pigment epithelium, lens, ciliary body and neural retina [108]. Its activity is required for the proper development of lens and optic cups [108]. The TF Meis2 is a main target of the developmental activity of microRNA-204. Aberrant expression of Meis2 released by microRNA-204 down-regulation leads to lens abnormalities, microphthalamia, and eye coloboma [108]. Interestingly, microRNA-204 and its host gene TRMP3 are transcriptionally co-regulated by the developmental TF PAX6 [109]. The analysis of genes aberrantly expressed in PAX6 mutants during eye development revealed that microRNA-204 target genes are highly represented. This led to identification of novel developmental target genes of microRNA-204, such as Sox11, a member of the SoxC family of neuronal TFs, which play a critical role in normal eye development. Intriguingly, PAX6 exerts its transcriptional program either modulating directly the expression of its targets genes, or indirectly modulating the expression of microRNA-204 that controls that of mRNA targets. This gives rise to a complex regulatory network that tunes and integrates coding and non-coding gene expression to pursue proper development.

Growing evidence indicates that microRNA-204 down-regulation is a common alteration in different types of human tumors. MicroRNA expression profiling of three different subsets of gastric cancer patients revealed that microRNA-204 expression was down-regulated in tumoral specimens when compared to matched peritumoral tissues [110]. TRPM3 gene loss was evidenced in a large fraction of the analysed gastric patients. This might consequently be one of the molecular

mechanisms underlying microRNA-204 down-regulation in human cancers [110-112]. Notably, gastric cancer patients can be grouped according to the extent of microRNA-204 down-regulation. Those characterized by a severe reduction (more than 0.5-fold) had the worst survival when compared to those with a mild downregulation of microRNA-204 (less than 0.5-fold) [110]. The pivotal anti-apoptotic protein, BCL-2, was shown to be a target of microRNA-204. Reduced expression of microRNA-204 paired with increased staining of BCL-2 in gastric cancer patients [110]. BCL-2 ectopic expression counteracted the pro-apoptotic role of microRNA-204 in response to 5-Fluorouracil [110]. Down-regulation of microRNA-204 in gastric cancers was also associated with increased expression of the class III histone deacetylase SIRT1. The targeting of SIRT1 by microRNA-204 ectopic expression favoured mesenchymal to epithelial transition (MET) phenotypes and suppressed anoikis resistance of gastric cancer cells [113]. Notably, network modelling performed in head and neck tumors by the combination of gene expression data with inheritable cancer traits and risk factor loci uncovered 18 targets of microRNA-204 that are mainly involved in the development of metastasis [114]. Interestingly, ectopic expression of microRNA-204 reduced the levels of its target genes and resulted in the inhibition of metastatic phenotype of head and neck cancer cell lines [114]. Altogether the localization of microRNA-204 lin the 9q21.1-q22.3 CAGR locus, a very well known risk factor locus for head and neck tumors and the identified target genes account for a bona-fide tumor suppressor micro-RNA. Down-regulation of microRNA-204-5p was also evidenced in endometrial carcinomas (EC), when compared to normal endometrium [115]. This might result from the aberrant expression of the neurotrophic receptor tyrosine kinase B (TrkB), which is a target of microRNA-204 [115]. Ectopic expression of TrkB led to accumulation of phospho-STAT3 that was recruited at a specific binding site of microRNA-204 host gene, TRMP3, and might account for microRNA-204 down-regulation in EC [115]. Loss of microRNA-204 expression through promoter methylation was evidenced in both glioma and neural stem cells [116]. This led to enhanced migration of glioma cells and to the acquisition of a stem cell-like phenotype. Indeed, attenuation of promoter methylation increased microRNA-204

expression in glioma cells and ectopic expression of microRNA-204 suppressed the tumorigenic potential of glioma cells. Mechanistic investigation revealed that microRNA-204 targets the expression of SOX4, a stemness TF, and EphB2, a receptor that promotes migration [116].

MicroRNA-204 is a transcriptional target of the von Hippel-Lindau tumor suppressor gene (VHL) that is lost in the largest fraction of clear cell renal cell carcinomas (ccRCC) [117]. VHL-induced expression of microRNA-204 resulted in the increased expression of short transcripts of TRMP3, thus indicating that microRNA-204 is not, at least in ccRCC, transcriptionally co-regulated with the large transcript encoding the full-length TRMP3 protein [117]. MicroRNA-204 expression is clearly reduced in ccRCC with known VHL status when compared to matched normal kidney samples and its reconstitution led to growth inhibition in vitro and in vivo [117]. The transcriptional tumor suppressor axis VHL/microRNA-204 inhibited macroautophagy. This occurs through the ability of microRNA-204 to directly target LC3B and VHL-induced expression of the parolog, LC3C that caused growth suppression.

It appears increasingly clear that both in development and in cancer the down-regulation of microRNA-204 disables coordinated transcriptional network and instigates unscheduled signalling pathways. These might contribute to developmental diseases or to aberrant proliferation, metastasis and poor response to conventional anticancer treatments. The identification of additional microRNA-204 targets and the dissection of the epigenetic events regulating its expression in normal and malignant tissues might provide intriguing insights, which would make microRNA-204 an appealing and hopefully druggable target.

**Perspectives and open questions**

Collectively, the reviewed groups of tumor suppressor microRNAs are emerging as major players of the cellular response to different types of oncogenic insults. Together with coding RNAs,

epigenetic modifications and other mechanisms, this response can lead to clonal expansion, spreading and chemoresistance of a given tumor. Along with ever improving understanding of the concerted alterations in microRNAs, many cardinal questions remain open. For example, it is logical that tumor suppressor microRNAs maintain complex crosstalks with coding tumor suppressor RNAs in order to fortify barriers to oncogenicity, but the details of this interplay are currently unknown. Also unknown are the molecular determinants underlying activation of intragenic and intergenic tumor suppressor microRNAs upon oncogenic insults. The multiplicity of RNA targets of each tumor suppressor microRNA poses a pivotal challenge. Moreover, it is conceivable that the tumor cell context influences target specificity, and consequently determines biological effects. These and additional issues will require deeper, perhaps more integrated understanding of tumor suppressor microRNAs.

**Legends to figures**

**Figure 1**. *Biogenesis and targets of microRNAs encoded by the miR-10b locus*. RNA polymerase II transcribes miRNA genes, generating long primary transcripts (pri miRNAs). Drosha-mediated cleavage of pri-miRNAs leads to the formation of a hairpin molecule, the pre-microRNA, that is exported to the cytoplasm by the exportin-5/RAN-GTP complex. In the cytoplasm, the pre-miRNA is cleaved by Dicer, to produce a ~22-nt RNA duplex. In the case of the mir-10b locus, these comprise two distinct 5' phosphorylated strands with 3′ overhangs, miR-10b-5p and miR-10b-3p. The functional strand of the duplex is incorporated into a multi-protein complex, the RNA-induced silencing complex (RISC), which regulates protein expression. In particular, in breast cancer functional miR-10b-3p targets BUB1, PLK1 and CCNA2 mRNAs, inducing a decrease in protein expression and a consequent inhibition of cancer cell proliferation.

**Figure 2.** *Let-7c regulation and functions.* The biogenesis of the let-7 family, including let-7c, is controlled by the RNA-binding proteins Lin28 and Lin28B. In particular, Lin28B inhibits Drosha-mediated formation of pre-let-7, as well as Dicer-mediated formation of let-7 duplexes. In turn let-7 family members downregulate expression of Lin28 and Lin28B, thereby establishing a negative feedback loop. Let-7c is downregulated in many types of tumors and its overexpression has a negative effect on cancer cells proliferation, metastasis and epithelial to mesenchymal transition.

**Figure 3**. *Macrophage-mediated miR-223 transfer*. (A) miR-223 is released by macrophages into microvesicles and it induces cellular differentiation of naive monocytes to increase in immune functions. (B) Macrophages mediate miR-223 transfer to cancer cells by mean of cell contact and gap junction communication. The transferred miR-223 is functionally active, and through decreased expression of Stathmin-1 (STMN1) and insulin-like growth factor-1 receptor (IGF-1R ), it induces growth arrest of cancer cells.

**Figure 4**. *miR-204 functions*. miR- 204 plays important roles in both eye development (A) and in tumor suppression (B). During eye development, high levels of miR-204 induce a reduction in Meis2 and Sox11 protein expression, thereby modulating the expression of other target proteins that contribute to normal development of the lens and retina. In particular, miR-204 expression is positively regulated by Pax6, a regulator of multiple processes during eye development, that in turn is activated by Meis2. Hence, miR-204 and Pax6 co-regulate each other via a negative feedback loop. miR-204 is downregulated in different types of cancer. Its tumor suppressor activity is mediated by the modulation of the expression of different oncoproteins depending on the cellular context.

**ACKNOWLEDGEMENTS**

Contribution of Associazione Italiana per la Ricerca sul Cancro-Rome Oncogenomic Center to GB, and of Epigen to GB was greatly appreciated.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**Bibliography**

1. Chin, L. & Gray, J. W. (2008) Translating insights from the cancer genome into clinical practice, *Nature.* **452**, 553-63.
2. Kloosterman, W. P. & Plasterk, R. H. (2006) The diverse functions of microRNAs in animal development and disease, *Dev Cell.* **11**, 441-50.
3. Calin, G. A., Sevignani, C., Dumitru, C. D., Hyslop, T., Noch, E., Yendamuri, S., Shimizu, M., Rattan, S., Bullrich, F., Negrini, M. & Croce, C. M. (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers, *Proc Natl Acad Sci U S A.* **101**, 2999-3004.
4. Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B. L., Mak, R. H., Ferrando, A. A., Downing, J. R., Jacks, T., Horvitz, H. R. & Golub, T. R. (2005) MicroRNA expression profiles classify human cancers, *Nature.* **435**, 834-8.
5. Sevignani, C., Calin, G. A., Nnadi, S. C., Shimizu, M., Davuluri, R. V., Hyslop, T., Demant, P., Croce, C. M. & Siracusa, L. D. (2007) MicroRNA genes are frequently located near mouse cancer susceptibility loci, *Proc Natl Acad Sci U S A.* **104**, 8017-22.
6. Lopez-Serra, P. & Esteller, M. (2012) DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer, *Oncogene.* **31**, 1609-22.
7. Melo, S. A. & Kalluri, R. (2012) Molecular pathways: microRNAs as cancer therapeutics, *Clin Cancer Res.* **18**, 4234-9.
8. Melo, S. A., Ropero, S., Moutinho, C., Aaltonen, L. A., Yamamoto, H., Calin, G. A., Rossi, S., Fernandez, A. F., Carneiro, F., Oliveira, C., Ferreira, B., Liu, C. G., Villanueva, A., Capella, G., Schwartz, S., Jr., Shiekhattar, R. & Esteller, M. (2009) A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function, *Nat Genet.* **41**, 365-70.
9. Melo, S. A., Moutinho, C., Ropero, S., Calin, G. A., Rossi, S., Spizzo, R., Fernandez, A. F., Davalos, V., Villanueva, A., Montoya, G., Yamamoto, H., Schwartz, S., Jr. & Esteller, M. (2010) A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells, *Cancer Cell.* **18**, 303-15.
10. Hill, J. M., Zhao, Y., Clement, C., Neumann, D. M. & Lukiw, W. J. (2009) HSV-1 infection of human brain cells induces miRNA-146a and Alzheimer-type inflammatory signaling, *Neuroreport.* **20**, 1500-5.
11. Rosenfeld, N., Aharonov, R., Meiri, E., Rosenwald, S., Spector, Y., Zepeniuk, M., Benjamin, H., Shabes, N., Tabak, S., Levy, A., Lebanony, D., Goren, Y., Silberschein, E., Targan, N., Ben-Ari, A., Gilad, S., Sion-Vardy, N., Tobar, A., Feinmesser, M., Kharenko, O., Nativ, O., Nass, D., Perelman, M., Yosepovich, A., Shalmon, B., Polak-Charcon, S., Fridman, E., Avniel, A., Bentwich, I., Bentwich, Z., Cohen, D., Chajut, A. & Barshack, I. (2008) MicroRNAs accurately identify cancer tissue origin, *Nat Biotechnol.* **26**, 462-9.
12. Biagioni, F., Bossel Ben-Moshe, N., Fontemaggi, G., Canu, V., Mori, F., Antoniani, B., Di Benedetto, A., Santoro, R., Germoni, S., De Angelis, F., Cambria, A., Avraham, R., Grasso, G.,

Strano, S., Muti, P., Mottolese, M., Yarden, Y., Domany, E. & Blandino, G. (2012) miR-10b*, a master inhibitor of the cell cycle, is down-regulated in human breast tumours, *EMBO Mol Med.* **4**, 1214-29.
13. Avraham, R., Sas-Chen, A., Manor, O., Steinfeld, I., Shalgi, R., Tarcic, G., Bossel, N., Zeisel, A., Amit, I., Zwang, Y., Enerly, E., Russnes, H. G., Biagioni, F., Mottolese, M., Strano, S., Blandino, G., Borresen-Dale, A. L., Pilpel, Y., Yakhini, Z., Segal, E. & Yarden, Y. (2010) EGF decreases the abundance of microRNAs that restrain oncogenic transcription factors, *Sci Signal.* **3**, ra43.
14. Rotllan, N. & Fernandez-Hernando, C. (2012) MicroRNA Regulation of Cholesterol Metabolism, *Cholesterol.* **2012**, 847849.
15. Davalos, A., Goedeke, L., Smibert, P., Ramirez, C. M., Warrier, N. P., Andreo, U., Cirera-Salinas, D., Rayner, K., Suresh, U., Pastor-Pareja, J. C., Esplugues, E., Fisher, E. A., Penalva, L. O., Moore, K. J., Suarez, Y., Lai, E. C. & Fernandez-Hernando, C. (2011) miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling, *Proc Natl Acad Sci U S A.* **108**, 9232-7.
16. Vasilescu, C., Rossi, S., Shimizu, M., Tudor, S., Veronese, A., Ferracin, M., Nicoloso, M. S., Barbarotto, E., Popa, M., Stanciulea, O., Fernandez, M. H., Tulbure, D., Bueso-Ramos, C. E., Negrini, M. & Calin, G. A. (2009) MicroRNA fingerprints identify miR-150 as a plasma prognostic marker in patients with sepsis, *PLoS One.* **4**, e7405.
17. Schmidt, W. M., Spiel, A. O., Jilma, B., Wolzt, M. & Muller, M. (2009) In vivo profile of the human leukocyte microRNA response to endotoxemia, *Biochem Biophys Res Commun.* **380**, 437-41.
18. Danger, R., Paul, C., Giral, M., Lavault, A., Foucher, Y., Degauque, N., Pallier, A., Durand, M., Castagnet, S., Duong Van Huyen, J. P., Delahousse, M., Renaudin, K., Soulillou, J. P. & Brouard, S. (2013) Expression of miR-142-5p in peripheral blood mononuclear cells from renal transplant patients with chronic antibody-mediated rejection, *PLoS One.* **8**, e60702.
19. Radom-Aizik, S., Zaldivar, F., Jr., Oliver, S., Galassetti, P. & Cooper, D. M. (2010) Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes, *J Appl Physiol (1985).* **109**, 252-61.
20. Rager, J. E., Smeester, L., Jaspers, I., Sexton, K. G. & Fry, R. C. (2011) Epigenetic changes induced by air toxics: formaldehyde exposure alters miRNA expression profiles in human lung cells, *Environ Health Perspect.* **119**, 494-500.
21. Elamin, B. K., Callegari, E., Gramantieri, L., Sabbioni, S. & Negrini, M. (2011) MicroRNA response to environmental mutagens in liver, *Mutat Res.* **717**, 67-76.
22. Izzotti, A., Calin, G. A., Arrigo, P., Steele, V. E., Croce, C. M. & De Flora, S. (2009) Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke, *FASEB J.* **23**, 806-12.
23. Biagioni, F., Bossel Ben-Moshe, N., Fontemaggi, G., Yarden, Y., Domany, E. & Blandino, G. (2013) The locus of microRNA-10b: a critical target for breast cancer insurgence and dissemination, *Cell Cycle.* **12**, 2371-5.
24. Iorio, M. V., Ferracin, M., Liu, C. G., Veronese, A., Spizzo, R., Sabbioni, S., Magri, E., Pedriali, M., Fabbri, M., Campiglio, M., Menard, S., Palazzo, J. P., Rosenberg, A., Musiani, P., Volinia, S., Nenci, I., Calin, G. A., Querzoli, P., Negrini, M. & Croce, C. M. (2005) MicroRNA gene expression deregulation in human breast cancer, *Cancer Res.* **65**, 7065-70.
25. Kim, K., Lee, H. C., Park, J. L., Kim, M., Kim, S. Y., Noh, S. M., Song, K. S., Kim, J. C. & Kim, Y. S. (2011) Epigenetic regulation of microRNA-10b and targeting of oncogenic MAPRE1 in gastric cancer, *Epigenetics.* **6**, 740-51.
26. Ma, L., Teruya-Feldstein, J. & Weinberg, R. A. (2007) Tumour invasion and metastasis initiated by microRNA-10b in breast cancer, *Nature.* **449**, 682-8.

27. Ma, L., Reinhardt, F., Pan, E., Soutschek, J., Bhat, B., Marcusson, E. G., Teruya-Feldstein, J., Bell, G. W. & Weinberg, R. A. (2010) Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model, *Nat Biotechnol.* **28**, 341-7.
28. Plummer, P. N., Freeman, R., Taft, R. J., Vider, J., Sax, M., Umer, B. A., Gao, D., Johns, C., Mattick, J. S., Wilton, S. D., Ferro, V., McMillan, N. A., Swarbrick, A., Mittal, V. & Mellick, A. S. (2013) MicroRNAs regulate tumor angiogenesis modulated by endothelial progenitor cells, *Cancer Res.* **73**, 341-52.
29. Lin, J., Teo, S., Lam, D. H., Jeyaseelan, K. & Wang, S. (2012) MicroRNA-10b pleiotropically regulates invasion, angiogenicity and apoptosis of tumor cells resembling mesenchymal subtype of glioblastoma multiforme, *Cell Death Dis.* **3**, e398.
30. Bossel Ben-Moshe, N., Avraham, R., Kedmi, M., Zeisel, A., Yitzhaky, A., Yarden, Y. & Domany, E. (2012) Context-specific microRNA analysis: identification of functional microRNAs and their mRNA targets, *Nucleic Acids Res.* **40**, 10614-27.
31. Pasquinelli, A. E., Reinhart, B. J., Slack, F., Martindale, M. Q., Kuroda, M. I., Maller, B., Hayward, D. C., Ball, E. E., Degnan, B., Muller, P., Spring, J., Srinivasan, A., Fishman, M., Finnerty, J., Corbo, J., Levine, M., Leahy, P., Davidson, E. & Ruvkun, G. (2000) Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA, *Nature.* **408**, 86-9.
32. Roush, S. & Slack, F. J. (2008) The let-7 family of microRNAs, *Trends in cell biology.* **18**, 505-16.
33. Reinhart, B. J., Slack, F. J., Basson, M., Pasquinelli, A. E., Bettinger, J. C., Rougvie, A. E., Horvitz, H. R. & Ruvkun, G. (2000) The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans, *Nature.* **403**, 901-6.
34. Wulczyn, F. G., Smirnova, L., Rybak, A., Brandt, C., Kwidzinski, E., Ninnemann, O., Strehle, M., Seiler, A., Schumacher, S. & Nitsch, R. (2007) Post-transcriptional regulation of the let-7 microRNA during neural cell specification, *FASEB J.* **21**, 415-26.
35. Yang, X., Rutnam, Z. J., Jiao, C., Wei, D., Xie, Y., Du, J., Zhong, L. & Yang, B. B. (2012) An anti-let-7 sponge decoys and decays endogenous let-7 functions, *Cell Cycle.* **11**, 3097-108.
36. Johnson, S. M., Grosshans, H., Shingara, J., Byrom, M., Jarvis, R., Cheng, A., Labourier, E., Reinert, K. L., Brown, D. & Slack, F. J. (2005) RAS is regulated by the let-7 microRNA family, *Cell.* **120**, 635-47.
37. Cui, S. Y., Huang, J. Y., Chen, Y. T., Song, H. Z., Feng, B., Huang, G. C., Wang, R., Chen, L. B. & De, W. (2013) Let-7c governs the acquisition of chemo- or radioresistance and epithelial-to-mesenchymal transition phenotypes in docetaxel-resistant lung adenocarcinoma, *Mol Cancer Res.* **11**, 699-713.
38. Christensen, B. C., Moyer, B. J., Avissar, M., Ouellet, L. G., Plaza, S. L., McClean, M. D., Marsit, C. J. & Kelsey, K. T. (2009) A let-7 microRNA-binding site polymorphism in the KRAS 3' UTR is associated with reduced survival in oral cancers, *Carcinogenesis.* **30**, 1003-7.
39. Zhang, W., Winder, T., Ning, Y., Pohl, A., Yang, D., Kahn, M., Lurje, G., Labonte, M. J., Wilson, P. M., Gordon, M. A., Hu-Lieskovan, S., Mauro, D. J., Langer, C., Rowinsky, E. K. & Lenz, H. J. (2011) A let-7 microRNA-binding site polymorphism in 3'-untranslated region of KRAS gene predicts response in wild-type KRAS patients with metastatic colorectal cancer treated with cetuximab monotherapy, *Ann Oncol.* **22**, 104-9.
40. Peng, Y., Laser, J., Shi, G., Mittal, K., Melamed, J., Lee, P. & Wei, J. J. (2008) Antiproliferative effects by Let-7 repression of high-mobility group A2 in uterine leiomyoma, *Mol Cancer Res.* **6**, 663-73.
41. Pelosi, A., Careccia, S., Lulli, V., Romania, P., Marziali, G., Testa, U., Lavorgna, S., Lo-Coco, F., Petti, M. C., Calabretta, B., Levrero, M., Piaggio, G. & Rizzo, M. G. (2013) miRNA let-7c promotes granulocytic differentiation in acute myeloid leukemia, *Oncogene.* **32**, 3648-54.
42. Tzur, G., Israel, A., Levy, A., Benjamin, H., Meiri, E., Shufaro, Y., Meir, K., Khvalevsky, E., Spector, Y., Rojansky, N., Bentwich, Z., Reubinoff, B. E. & Galun, E. (2009) Comprehensive gene

and microRNA expression profiling reveals a role for microRNAs in human liver development, *PLoS One.* **4**, e7511.
43. Han, H. B., Gu, J., Zuo, H. J., Chen, Z. G., Zhao, W., Li, M., Ji, D. B., Lu, Y. Y. & Zhang, Z. Q. (2012) Let-7c functions as a metastasis suppressor by targeting MMP11 and PBX3 in colorectal cancer, *J Pathol.* **226**, 544-55.
44. Nadiminty, N., Tummala, R., Lou, W., Zhu, Y., Shi, X. B., Zou, J. X., Chen, H., Zhang, J., Chen, X., Luo, J., deVere White, R. W., Kung, H. J., Evans, C. P. & Gao, A. C. (2012) MicroRNA let-7c is downregulated in prostate cancer and suppresses prostate cancer growth, *PLoS One.* **7**, e32832.
45. Shah, Y. M., Morimura, K., Yang, Q., Tanabe, T., Takagi, M. & Gonzalez, F. J. (2007) Peroxisome proliferator-activated receptor alpha regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation, *Mol Cell Biol.* **27**, 4238-47.
46. Zhao, B., Han, H., Chen, J., Zhang, Z., Li, S., Fang, F., Zheng, Q., Ma, Y., Zhang, J., Wu, N. & Yang, Y. (2014) MicroRNA let-7c inhibits migration and invasion of human non-small cell lung cancer by targeting ITGB3 and MAP4K3, *Cancer Lett.* **342**, 43-51.
47. Yamada, H., Yanagisawa, K., Tokumaru, S., Taguchi, A., Nimura, Y., Osada, H., Nagino, M. & Takahashi, T. (2008) Detailed characterization of a homozygously deleted region corresponding to a candidate tumor suppressor locus at 21q11-21 in human lung cancer, *Genes Chromosomes Cancer.* **47**, 810-8.
48. Piskounova, E., Polytarchou, C., Thornton, J. E., LaPierre, R. J., Pothoulakis, C., Hagan, J. P., Iliopoulos, D. & Gregory, R. I. (2011) Lin28A and Lin28B inhibit let-7 microRNA biogenesis by distinct mechanisms, *Cell.* **147**, 1066-79.
49. Heo, I., Joo, C., Cho, J., Ha, M., Han, J. & Kim, V. N. (2008) Lin28 mediates the terminal uridylation of let-7 precursor MicroRNA, *Molecular cell.* **32**, 276-84.
50. Heo, I., Joo, C., Kim, Y. K., Ha, M., Yoon, M. J., Cho, J., Yeom, K. H., Han, J. & Kim, V. N. (2009) TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation, *Cell.* **138**, 696-708.
51. Thornton, J. E. & Gregory, R. I. (2012) How does Lin28 let-7 control development and disease?, *Trends Cell Biol.* **22**, 474-82.
52. Guo, Y., Chen, Y., Ito, H., Watanabe, A., Ge, X., Kodama, T. & Aburatani, H. (2006) Identification and characterization of lin-28 homolog B (LIN28B) in human hepatocellular carcinoma, *Gene.* **384**, 51-61.
53. Moss, E. G. & Tang, L. (2003) Conservation of the heterochronic regulator Lin-28, its developmental expression and microRNA complementary sites, *Developmental biology.* **258**, 432-42.
54. Nadiminty, N., Tummala, R., Lou, W., Zhu, Y., Zhang, J., Chen, X., eVere White, R. W., Kung, H. J., Evans, C. P. & Gao, A. C. (2012) MicroRNA let-7c suppresses androgen receptor expression and activity via regulation of Myc expression in prostate cancer cells, *J Biol Chem.* **287**, 1527-37.
55. Wang, P. Y., Sun, Y. X., Zhang, S., Pang, M., Zhang, H. H., Gao, S. Y., Zhang, C., Lv, C. J. & Xie, S. Y. (2013) Let-7c inhibits A549 cell proliferation through oncogenic TRIB2 related factors, *FEBS Lett.* **587**, 2675-81.
56. Thiery, J. P., Acloque, H., Huang, R. Y. & Nieto, M. A. (2009) Epithelial-mesenchymal transitions in development and disease, *Cell.* **139**, 871-90.
57. Chen, C. Z., Li, L., Lodish, H. F. & Bartel, D. P. (2004) MicroRNAs modulate hematopoietic lineage differentiation, *Science.* **303**, 83-6.
58. Fazi, F., Rosa, A., Fatica, A., Gelmetti, V., De Marchis, M. L., Nervi, C. & Bozzoni, I. (2005) A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPalpha regulates human granulopoiesis, *Cell.* **123**, 819-31.

59. Vian, L., Di Carlo, M., Pelosi, E., Fazi, F., Santoro, S., Cerio, A. M., Boe, A., Rotilio, V., Billi, M., Racanicchi, S., Testa, U., Grignani, F. & Nervi, C. (2013) Transcriptional fine-tuning of microRNA-223 levels directs lineage choice of human hematopoietic progenitors, *Cell death and differentiation*.
60. Nervi, C., Fazi, F., Rosa, A., Fatica, A. & Bozzoni, I. (2007) Emerging role for microRNAs in acute promyelocytic leukemia, *Current topics in microbiology and immunology.* **313**, 73-84.
61. Zardo, G., Ciolfi, A., Vian, L., Starnes, L. M., Billi, M., Racanicchi, S., Maresca, C., Fazi, F., Travaglini, L., Noguera, N., Mancini, M., Nanni, M., Cimino, G., Lo-Coco, F., Grignani, F. & Nervi, C. (2012) Polycombs and microRNA-223 regulate human granulopoiesis by transcriptional control of target gene expression, *Blood.* **119**, 4034-46.
62. Fazi, F., Racanicchi, S., Zardo, G., Starnes, L. M., Mancini, M., Travaglini, L., Diverio, D., Ammatuna, E., Cimino, G., Lo-Coco, F., Grignani, F. & Nervi, C. (2007) Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein, *Cancer cell.* **12**, 457-66.
63. Pulikkan, J. A., Dengler, V., Peramangalam, P. S., Peer Zada, A. A., Muller-Tidow, C., Bohlander, S. K., Tenen, D. G. & Behre, G. (2010) Cell-cycle regulator E2F1 and microRNA-223 comprise an autoregulatory negative feedback loop in acute myeloid leukemia, *Blood.* **115**, 1768-78.
64. Valadi, H., Ekstrom, K., Bossios, A., Sjostrand, M., Lee, J. J. & Lotvall, J. O. (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, *Nature cell biology.* **9**, 654-9.
65. Ismail, N., Wang, Y., Dakhlallah, D., Moldovan, L., Agarwal, K., Batte, K., Shah, P., Wisler, J., Eubank, T. D., Tridandapani, S., Paulaitis, M. E., Piper, M. G. & Marsh, C. B. (2013) Macrophage microvesicles induce macrophage differentiation and miR-223 transfer, *Blood.* **121**, 984-95.
66. Aucher, A., Rudnicka, D. & Davis, D. M. (2013) MicroRNAs Transfer from Human Macrophages to Hepato-Carcinoma Cells and Inhibit Proliferation, *Journal of immunology.* **191**, 6250-60.
67. Wong, Q. W., Lung, R. W., Law, P. T., Lai, P. B., Chan, K. Y., To, K. F. & Wong, N. (2008) MicroRNA-223 is commonly repressed in hepatocellular carcinoma and potentiates expression of Stathmin1, *Gastroenterology.* **135**, 257-69.
68. Jia, C. Y., Li, H. H., Zhu, X. C., Dong, Y. W., Fu, D., Zhao, Q. L., Wu, W. & Wu, X. Z. (2011) MiR-223 suppresses cell proliferation by targeting IGF-1R, *PloS one.* **6**, e27008.
69. Masciarelli, S., Fontemaggi, G., Di Agostino, S., Donzelli, S., Carcarino, E., Strano, S. & Blandino, G. (2013) Gain-of-function mutant p53 downregulates miR-223 contributing to chemoresistance of cultured tumor cells, *Oncogene*.
70. Nian, W., Ao, X., Wu, Y., Huang, Y., Shao, J., Wang, Y., Chen, Z., Chen, F. & Wang, D. (2013) miR-223 functions as a potent tumor suppressor of the Lewis lung carcinoma cell line by targeting insulin-like growth factor-1 receptor and cyclin-dependent kinase 2, *Oncology letters.* **6**, 359-366.
71. Wu, L., Li, H., Jia, C. Y., Cheng, W., Yu, M., Peng, M., Zhu, Y., Zhao, Q., Dong, Y. W., Shao, K., Wu, A. & Wu, X. Z. (2012) MicroRNA-223 regulates FOXO1 expression and cell proliferation, *FEBS letters.* **586**, 1038-43.
72. Mansour, M. R., Sanda, T., Lawton, L. N., Li, X., Kreslavsky, T., Novina, C. D., Brand, M., Gutierrez, A., Kelliher, M. A., Jamieson, C. H., von Boehmer, H., Young, R. A. & Look, A. T. (2013) The TAL1 complex targets the FBXW7 tumor suppressor by activating miR-223 in human T cell acute lymphoblastic leukemia, *The Journal of experimental medicine.* **210**, 1545-57.
73. Mavrakis, K. J., Van Der Meulen, J., Wolfe, A. L., Liu, X., Mets, E., Taghon, T., Khan, A. A., Setty, M., Rondou, P., Vandenberghe, P., Delabesse, E., Benoit, Y., Socci, N. B., Leslie, C. S., Van Vlierberghe, P., Speleman, F. & Wendel, H. G. (2011) A cooperative microRNA-tumor

suppressor gene network in acute T-cell lymphoblastic leukemia (T-ALL), *Nature genetics.* **43**, 673-8.
74. Iio, A., Nakagawa, Y., Hirata, I., Naoe, T. & Akao, Y. (2010) Identification of non-coding RNAs embracing microRNA-143/145 cluster, *Mol Cancer.* **9**, 136.
75. Michael, M. Z., SM, O. C., van Holst Pellekaan, N. G., Young, G. P. & James, R. J. (2003) Reduced accumulation of specific microRNAs in colorectal neoplasia, *Molecular cancer research : MCR.* **1**, 882-91.
76. Starczynowski, D. T., Kuchenbauer, F., Argiropoulos, B., Sung, S., Morin, R., Muranyi, A., Hirst, M., Hogge, D., Marra, M., Wells, R. A., Buckstein, R., Lam, W., Humphries, R. K. & Karsan, A. (2010) Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype, *Nature medicine.* **16**, 49-58.
77. Sachdeva, M., Zhu, S., Wu, F., Wu, H., Walia, V., Kumar, S., Elble, R., Watabe, K. & Mo, Y. Y. (2009) p53 represses c-Myc through induction of the tumor suppressor miR-145, *Proceedings of the National Academy of Sciences of the United States of America.* **106**, 3207-12.
78. Suh, S. O., Chen, Y., Zaman, M. S., Hirata, H., Yamamura, S., Shahryari, V., Liu, J., Tabatabai, Z. L., Kakar, S., Deng, G., Tanaka, Y. & Dahiya, R. (2011) MicroRNA-145 is regulated by DNA methylation and p53 gene mutation in prostate cancer, *Carcinogenesis.* **32**, 772-8.
79. Sachdeva, M., Liu, Q., Cao, J., Lu, Z. & Mo, Y. Y. (2012) Negative regulation of miR-145 by C/EBP-beta through the Akt pathway in cancer cells, *Nucleic acids research.* **40**, 6683-92.
80. Suzuki, H. I., Yamagata, K., Sugimoto, K., Iwamoto, T., Kato, S. & Miyazono, K. (2009) Modulation of microRNA processing by p53, *Nature.* **460**, 529-33.
81. Iio, A., Takagi, T., Miki, K., Naoe, T., Nakayama, A. & Akao, Y. (2013) DDX6 post-transcriptionally down-regulates miR-143/145 expression through host gene NCR143/145 in cancer cells, *Biochimica et biophysica acta.* **1829**, 1102-10.
82. Sachdeva, M. & Mo, Y. Y. (2010) MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1, *Cancer research.* **70**, 378-87.
83. Gotte, M., Mohr, C., Koo, C. Y., Stock, C., Vaske, A. K., Viola, M., Ibrahim, S. A., Peddibhotla, S., Teng, Y. H., Low, J. Y., Ebnet, K., Kiesel, L. & Yip, G. W. (2010) miR-145-dependent targeting of junctional adhesion molecule A and modulation of fascin expression are associated with reduced breast cancer cell motility and invasiveness, *Oncogene.* **29**, 6569-80.
84. Zhang, H., Pu, J., Qi, T., Qi, M., Yang, C., Li, S., Huang, K., Zheng, L. & Tong, Q. (2012) MicroRNA-145 inhibits the growth, invasion, metastasis and angiogenesis of neuroblastoma cells through targeting hypoxia-inducible factor 2 alpha, *Oncogene*.
85. Gao, P., Xing, A. Y., Zhou, G. Y., Zhang, T. G., Zhang, J. P., Gao, C., Li, H. & Shi, D. B. (2013) The molecular mechanism of microRNA-145 to suppress invasion-metastasis cascade in gastric cancer, *Oncogene.* **32**, 491-501.
86. Fuse, M., Nohata, N., Kojima, S., Sakamoto, S., Chiyomaru, T., Kawakami, K., Enokida, H., Nakagawa, M., Naya, Y., Ichikawa, T. & Seki, N. (2011) Restoration of miR-145 expression suppresses cell proliferation, migration and invasion in prostate cancer by targeting FSCN1, *International journal of oncology.* **38**, 1093-101.
87. Kliese, N., Gobrecht, P., Pachow, D., Andrae, N., Wilisch-Neumann, A., Kirches, E., Riek-Burchardt, M., Angenstein, F., Reifenberger, G., Riemenschneider, M. J., Meese, E., Panayotova-Dimitrova, D., Gutmann, D. H. & Mawrin, C. (2013) miRNA-145 is downregulated in atypical and anaplastic meningiomas and negatively regulates motility and proliferation of meningioma cells, *Oncogene.* **32**, 4712-20.
88. Shi, B., Sepp-Lorenzino, L., Prisco, M., Linsley, P., deAngelis, T. & Baserga, R. (2007) Micro RNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells, *The Journal of biological chemistry.* **282**, 32582-90.
89. Gregersen, L. H., Jacobsen, A. B., Frankel, L. B., Wen, J., Krogh, A. & Lund, A. H. (2010) MicroRNA-145 targets YES and STAT1 in colon cancer cells, *PloS one.* **5**, e8836.

90. Xu, Q., Liu, L. Z., Qian, X., Chen, Q., Jiang, Y., Li, D., Lai, L. & Jiang, B. H. (2012) MiR-145 directly targets p70S6K1 in cancer cells to inhibit tumor growth and angiogenesis, *Nucleic acids research.* **40**, 761-74.
91. Cho, W. C., Chow, A. S. & Au, J. S. (2011) MiR-145 inhibits cell proliferation of human lung adenocarcinoma by targeting EGFR and NUDT1, *RNA biology.* **8**, 125-31.
92. Yanaihara, N., Caplen, N., Bowman, E., Seike, M., Kumamoto, K., Yi, M., Stephens, R. M., Okamoto, A., Yokota, J., Tanaka, T., Calin, G. A., Liu, C. G., Croce, C. M. & Harris, C. C. (2006) Unique microRNA molecular profiles in lung cancer diagnosis and prognosis, *Cancer cell.* **9**, 189-98.
93. Zou, C., Xu, Q., Mao, F., Li, D., Bian, C., Liu, L. Z., Jiang, Y., Chen, X., Qi, Y., Zhang, X., Wang, X., Sun, Q., Kung, H. F., Lin, M. C., Dress, A., Wardle, F., Jiang, B. H. & Lai, L. (2012) MiR-145 inhibits tumor angiogenesis and growth by N-RAS and VEGF, *Cell cycle.* **11**, 2137-45.
94. Wang, S., Bian, C., Yang, Z., Bo, Y., Li, J., Zeng, L., Zhou, H. & Zhao, R. C. (2009) miR-145 inhibits breast cancer cell growth through RTKN, *International journal of oncology.* **34**, 1461-6.
95. Spizzo, R., Nicoloso, M. S., Lupini, L., Lu, Y., Fogarty, J., Rossi, S., Zagatti, B., Fabbri, M., Veronese, A., Liu, X., Davuluri, R., Croce, C. M., Mills, G., Negrini, M. & Calin, G. A. (2010) miR-145 participates with TP53 in a death-promoting regulatory loop and targets estrogen receptor-alpha in human breast cancer cells, *Cell Death Differ.* **17**, 246-54.
96. Zhang, J., Sun, Q., Zhang, Z., Ge, S., Han, Z. G. & Chen, W. T. (2013) Loss of microRNA-143/145 disturbs cellular growth and apoptosis of human epithelial cancers by impairing the MDM2-p53 feedback loop, *Oncogene.* **32**, 61-9.
97. Polytarchou, C., Iliopoulos, D. & Struhl, K. (2012) An integrated transcriptional regulatory circuit that reinforces the breast cancer stem cell state, *Proceedings of the National Academy of Sciences of the United States of America.* **109**, 14470-5.
98. Xu, N., Papagiannakopoulos, T., Pan, G., Thomson, J. A. & Kosik, K. S. (2009) MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells, *Cell.* **137**, 647-58.
99. Cioce, M., Ganci, F., Canu, V., Sacconi, A., Mori, F., Canino, C., Korita, E., Casini, B., Alessandrini, G., Cambria, A., Carosi, M. A., Blandino, R., Panebianco, V., Facciolo, F., Visca, P., Volinia, S., Muti, P., Strano, S., Croce, C. M., Pass, H. I. & Blandino, G. (2013) Protumorigenic effects of mir-145 loss in malignant pleural mesothelioma, *Oncogene*.
100. Kent, O. A., Chivukula, R. R., Mullendore, M., Wentzel, E. A., Feldmann, G., Lee, K. H., Liu, S., Leach, S. D., Maitra, A. & Mendell, J. T. (2010) Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway, *Genes & development.* **24**, 2754-9.
101. Kent, O. A., Fox-Talbot, K. & Halushka, M. K. (2013) RREB1 repressed miR-143/145 modulates KRAS signaling through downregulation of multiple targets, *Oncogene.* **32**, 2576-85.
102. Pagliuca, A., Valvo, C., Fabrizi, E., di Martino, S., Biffoni, M., Runci, D., Forte, S., De Maria, R. & Ricci-Vitiani, L. (2013) Analysis of the combined action of miR-143 and miR-145 on oncogenic pathways in colorectal cancer cells reveals a coordinate program of gene repression, *Oncogene.* **32**, 4806-13.
103. Chen, X., Gong, J., Zeng, H., Chen, N., Huang, R., Huang, Y., Nie, L., Xu, M., Xia, J., Zhao, F., Meng, W. & Zhou, Q. (2010) MicroRNA145 targets BNIP3 and suppresses prostate cancer progression, *Cancer research.* **70**, 2728-38.
104. Ren, D., Wang, M., Guo, W., Zhao, X., Tu, X., Huang, S., Zou, X. & Peng, X. (2013) Wild-type p53 suppresses the epithelial-mesenchymal transition and stemness in PC-3 prostate cancer cells by modulating miR145, *International journal of oncology.* **42**, 1473-81.

105. Hart, M., Wach, S., Nolte, E., Szczyrba, J., Menon, R., Taubert, H., Hartmann, A., Stoehr, R., Wieland, W., Grasser, F. A. & Wullich, B. (2013) The proto-oncogene ERG is a target of microRNA miR-145 in prostate cancer, *The FEBS journal.* **280**, 2105-16.
106. Wang, F. E., Zhang, C., Maminishkis, A., Dong, L., Zhi, C., Li, R., Zhao, J., Majerciak, V., Gaur, A. B., Chen, S. & Miller, S. S. (2010) MicroRNA-204/211 alters epithelial physiology, *FASEB J.* **24**, 1552-71.
107. Oberwinkler, J. (2007) TRPM3, a biophysical enigma?, *Biochem Soc Trans.* **35**, 89-90.
108. Conte, I., Carrella, S., Avellino, R., Karali, M., Marco-Ferreres, R., Bovolenta, P. & Banfi, S. (2010) miR-204 is required for lens and retinal development via Meis2 targeting, *Proc Natl Acad Sci U S A.* **107**, 15491-6.
109. Shaham, O., Gueta, K., Mor, E., Oren-Giladi, P., Grinberg, D., Xie, Q., Cvekl, A., Shomron, N., Davis, N., Keydar-Prizant, M., Raviv, S., Pasmanik-Chor, M., Bell, R. E., Levy, C., Avellino, R., Banfi, S., Conte, I. & Ashery-Padan, R. (2013) Pax6 regulates gene expression in the vertebrate lens through miR-204, *PLoS Genet.* **9**, e1003357.
110. Sacconi, A., Biagioni, F., Canu, V., Mori, F., Di Benedetto, A., Lorenzon, L., Ercolani, C., Di Agostino, S., Cambria, A. M., Germoni, S., Grasso, G., Blandino, R., Panebianco, V., Ziparo, V., Federici, O., Muti, P., Strano, S., Carboni, F., Mottolese, M., Diodoro, M., Pescarmona, E., Garofalo, A. & Blandino, G. (2012) miR-204 targets Bcl-2 expression and enhances responsiveness of gastric cancer, *Cell Death Dis.* **3**, e423.
111. Staub, E., Grone, J., Mennerich, D., Ropcke, S., Klamann, I., Hinzmann, B., Castanos-Velez, E., Mann, B., Pilarsky, C., Brummendorf, T., Weber, B., Buhr, H. J. & Rosenthal, A. (2006) A genome-wide map of aberrantly expressed chromosomal islands in colorectal cancer, *Mol Cancer.* **5**, 37.
112. Balachandar, V., Lakshman Kumar, B., Sasikala, K., Manikantan, P., Sangeetha, R. & Mohana Devi, S. (2007) Identification of a high frequency of chromosomal rearrangements in the centromeric regions of prostate cancer patients, *J Zhejiang Univ Sci B.* **8**, 638-46.
113. Zhang, L., Wang, X. & Chen, P. (2013) MiR-204 down regulates SIRT1 and reverts SIRT1-induced epithelial-mesenchymal transition, anoikis resistance and invasion in gastric cancer cells, *BMC Cancer.* **13**, 290.
114. Lee, Y., Yang, X., Huang, Y., Fan, H., Zhang, Q., Wu, Y., Li, J., Hasina, R., Cheng, C., Lingen, M. W., Gerstein, M. B., Weichselbaum, R. R., Xing, H. R. & Lussier, Y. A. (2010) Network modeling identifies molecular functions targeted by miR-204 to suppress head and neck tumor metastasis, *PLoS Comput Biol.* **6**, e1000730.
115. Bao, W., Wang, H. H., Tian, F. J., He, X. Y., Qiu, M. T., Wang, J. Y., Zhang, H. J., Wang, L. H. & Wan, X. P. (2013) A TrkB-STAT3-miR-204-5p regulatory circuitry controls proliferation and invasion of endometrial carcinoma cells, *Mol Cancer.* **12**, 155.
116. Ying, Z., Li, Y., Wu, J., Zhu, X., Yang, Y., Tian, H., Li, W., Hu, B., Cheng, S. Y. & Li, M. (2013) Loss of miR-204 expression enhances glioma migration and stem cell-like phenotype, *Cancer Res.* **73**, 990-9.
117. Mikhaylova, O., Stratton, Y., Hall, D., Kellner, E., Ehmer, B., Drew, A. F., Gallo, C. A., Plas, D. R., Biesiada, J., Meller, J. & Czyzyk-Krzeska, M. F. (2012) VHL-regulated MiR-204 suppresses tumor growth through inhibition of LC3B-mediated autophagy in renal clear cell carcinoma, *Cancer Cell.* **21**, 532-46.